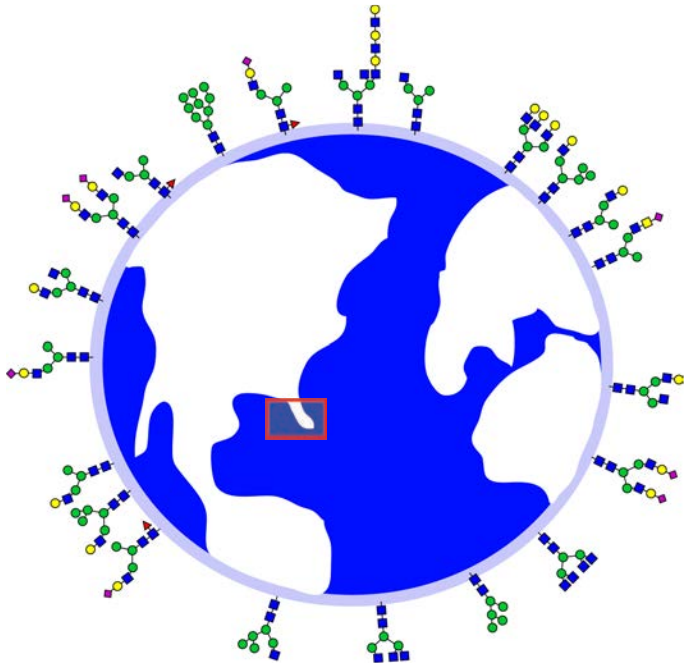
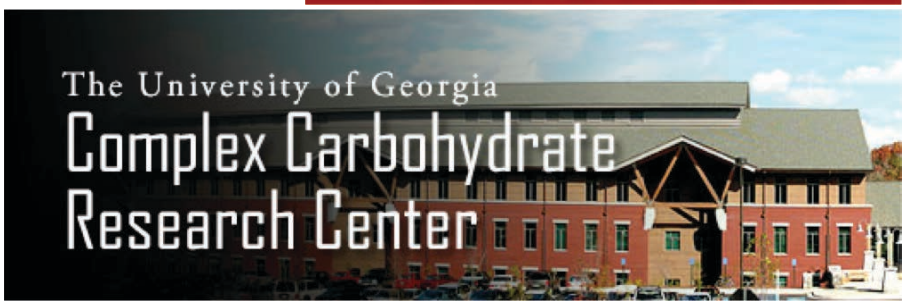
Strategies and tools for comprehensive glycomics

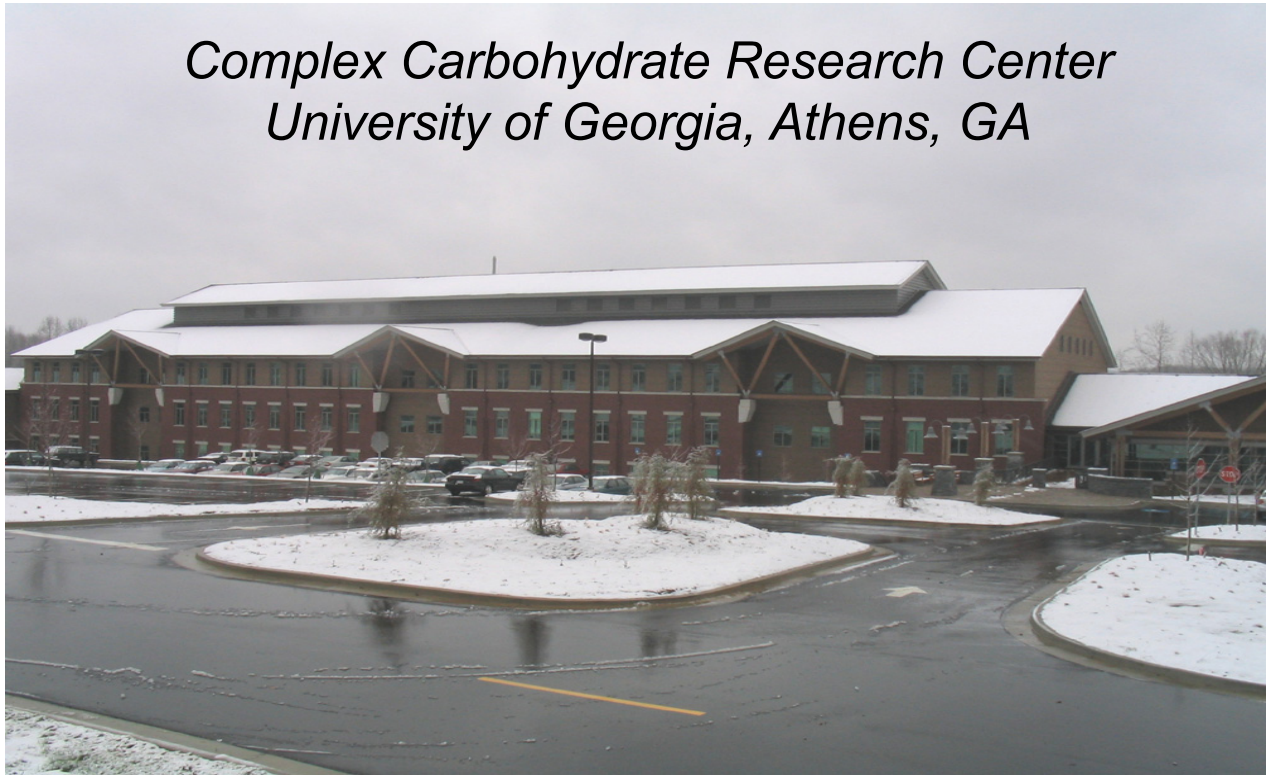


Complex Carbohydrate Research Center
University of Georgia

Kazuhiro Aoki

International Life Science
Integration Workshop
Tokyo, Japan
March 5, 2018

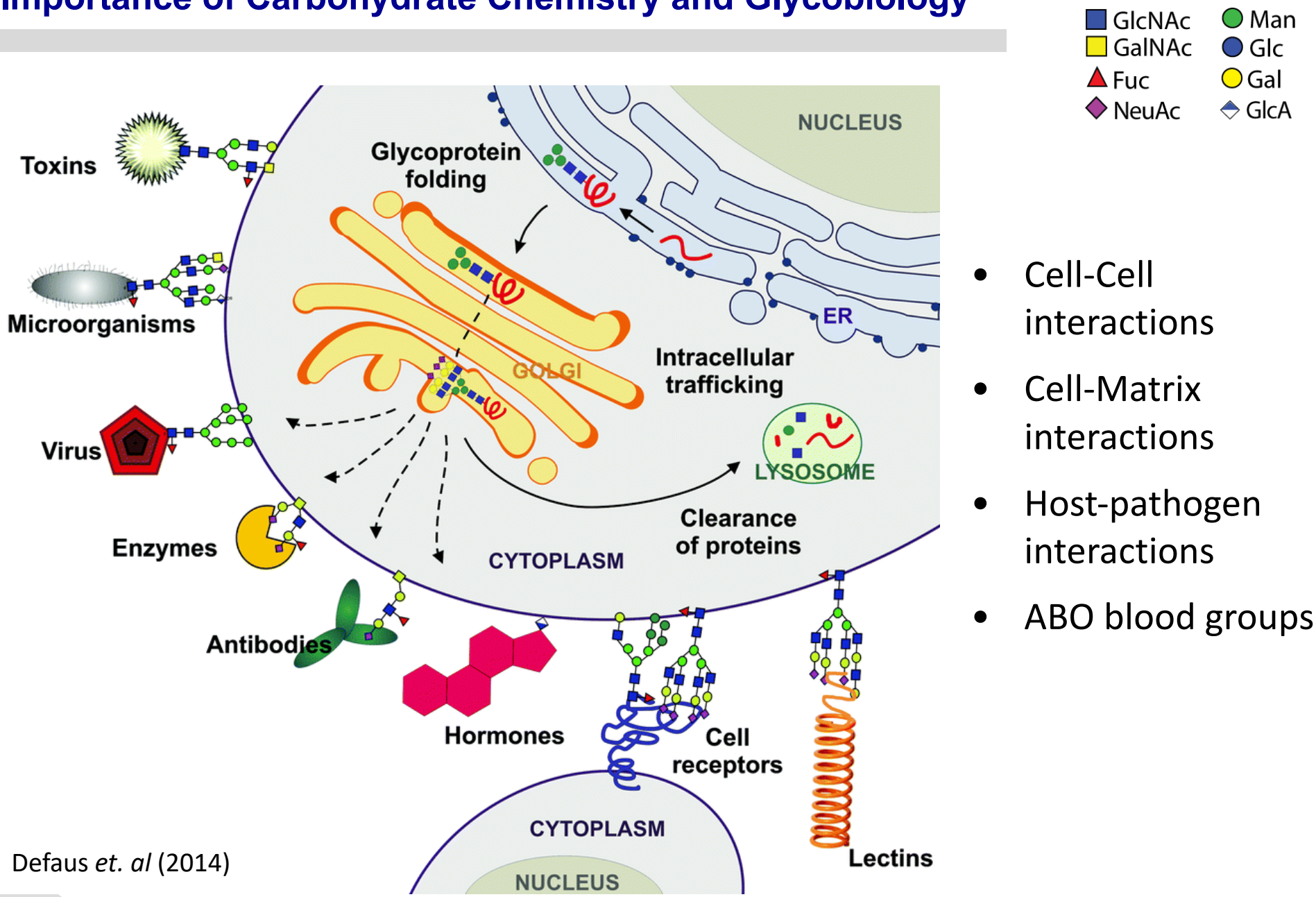




- 140,000 sq ft. facility; state-of-the-art MS, computing and NMR instrumentation
- 18 Principal Investigators, over 300 total members (staff, students, post-docs, etc.)
- Approximately 20 million US dollars in direct cost each year
- Functions of glycans in biomedical applications, animal and plant physiology, microbial pathogenesis, etc.
- Strong emphasis on studying STRUCTURE and FUNCTION

www.ccrc.uga.edu

Importance of Carbohydrate Chemistry and Glycobiology

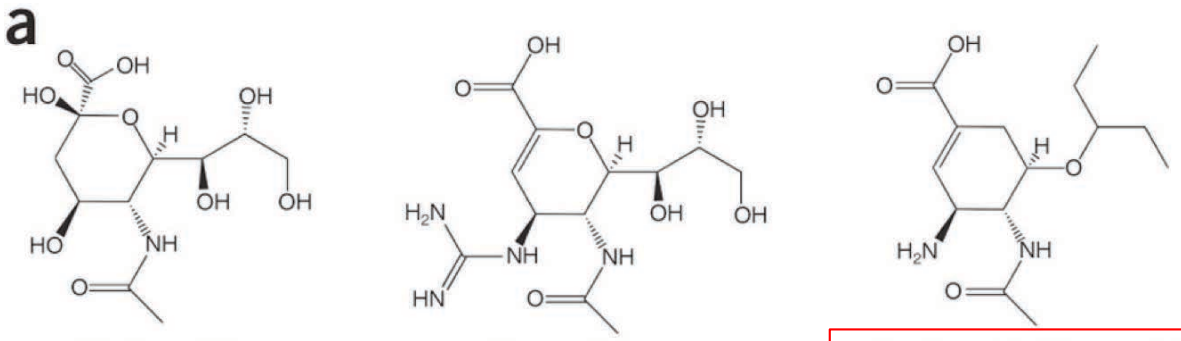


- Cell-Cell interactions
- Cell-Matrix interactions
- Host-pathogen interactions
- ABO blood groups

Defaus *et. al* (2014)

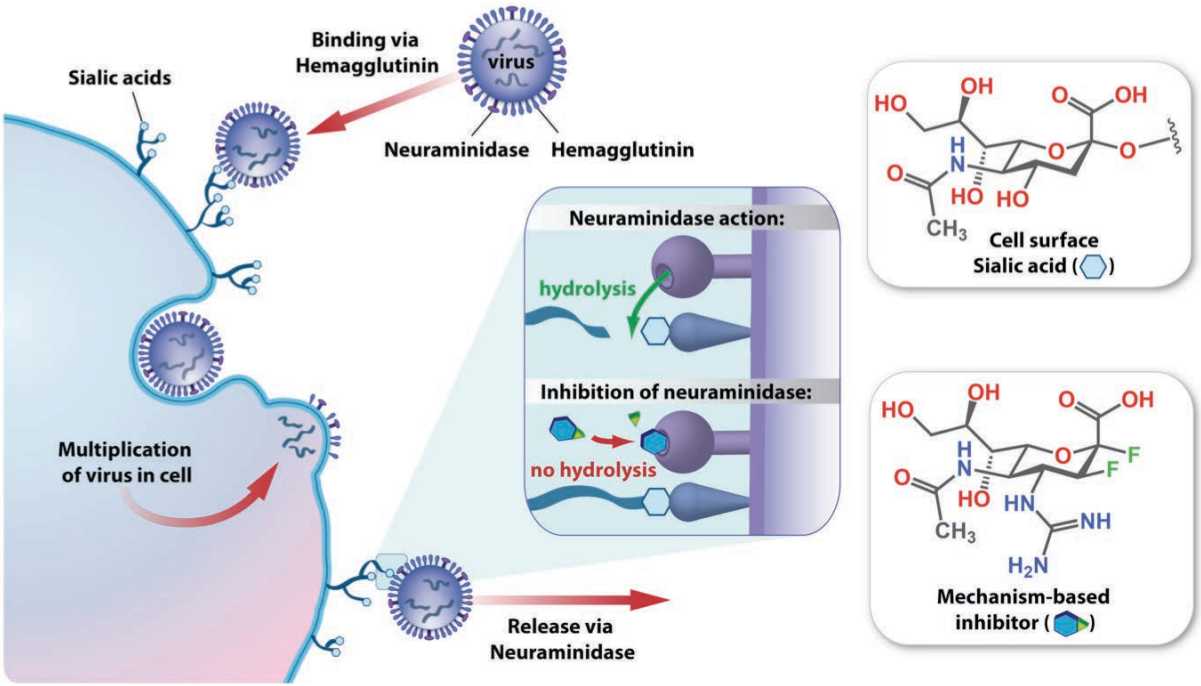
Courtesy of Dr. Simone Kurz

Binding of sialic acid–mimic drugs zanamivir (Relenza) and oseltamivir (Tamiflu) to neuraminidase (NA).



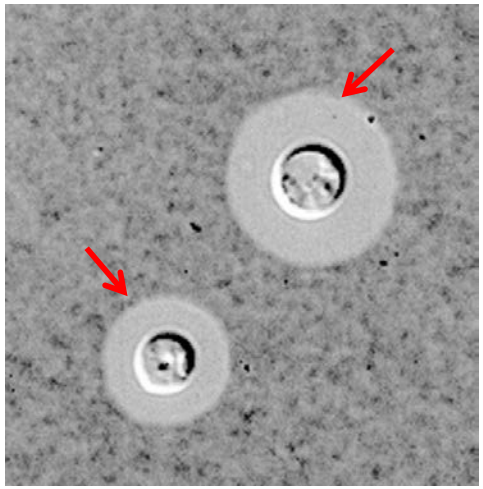
structural mimics of sialic acid

Flu virus

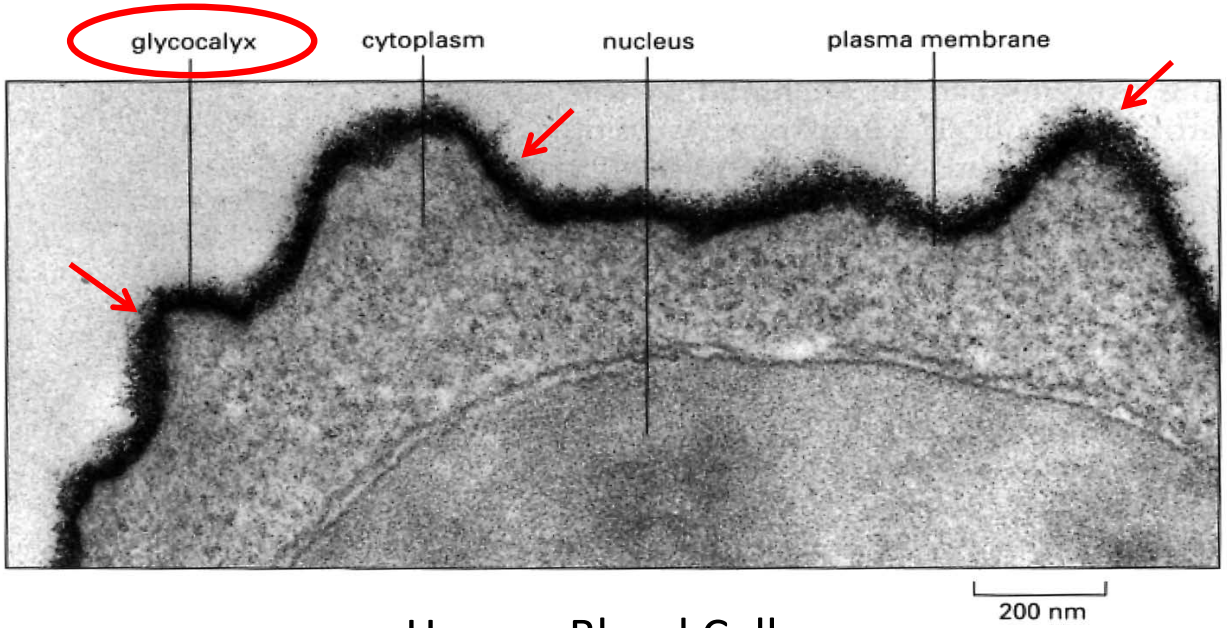


Nature Structural & Molecular Biology 17, 530–538 (2010)

All living organisms produce complex carbohydrates of one type or another



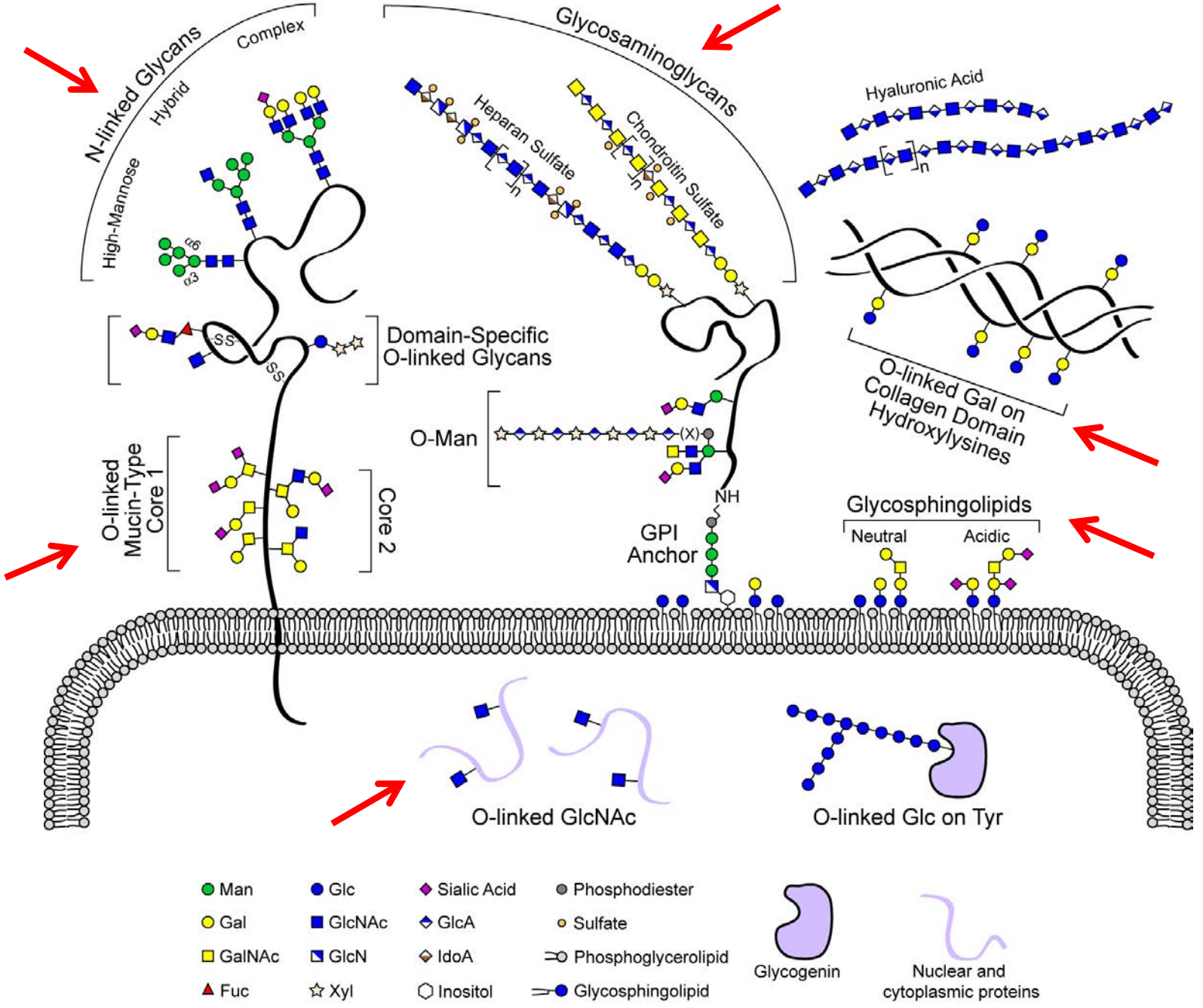
Fungus



Human Blood Cell

- Also referred to as glycans or glycoconjugates, they are essential for many biological functions that occur at the surface of cells, especially protection, signaling, and recognition.
- Gangliosides, like GM3, are one type of glycoconjugate produced by animal cells.
- Glycoconjugates are very diverse in their structure.

Glycodiversity in Animal



Linking glycomics to function

Glycomics

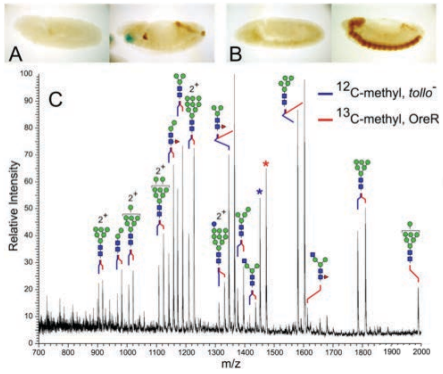
Glycan library

Glycan microarray

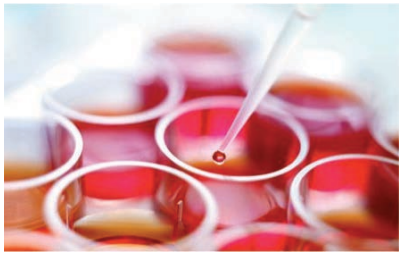
Bioinfomatics

Molecular modeling

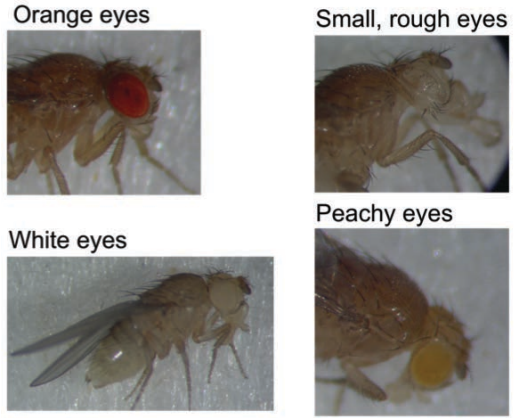
Synthetic Chemistry



Biochemical approach



Genetic approach



Glycobiology

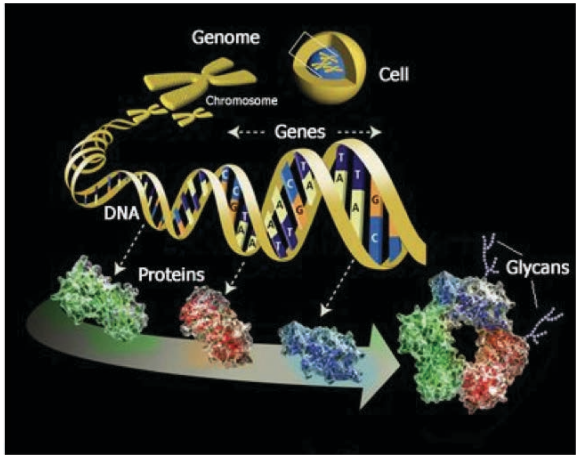
Method development

MS spec.

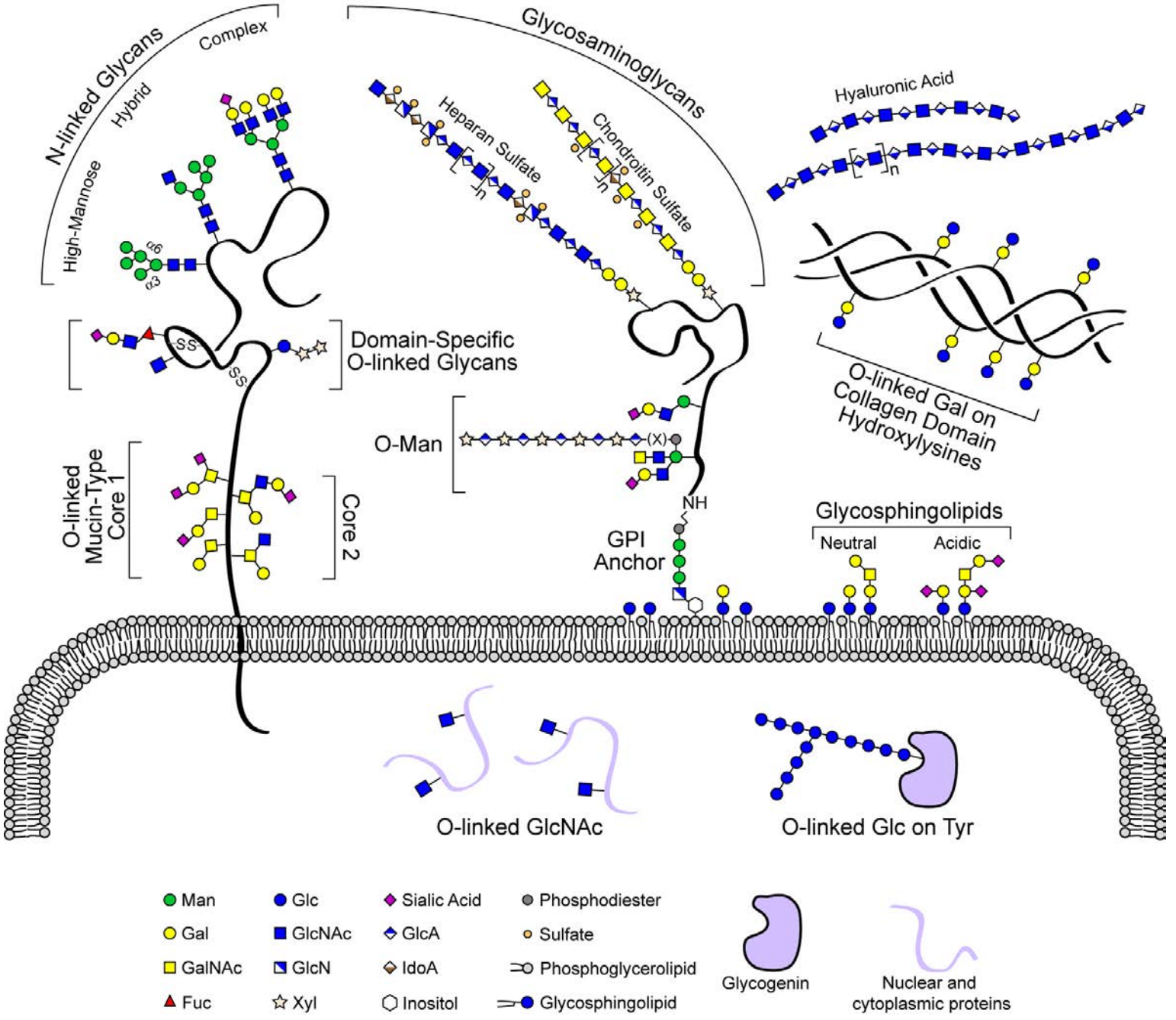
HPLC

Glycoproteomics

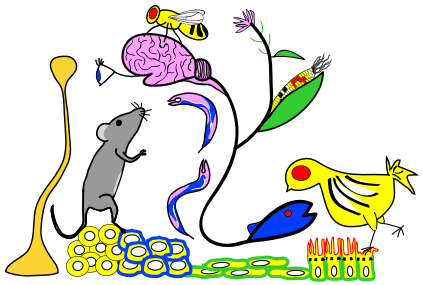
Biological approach



Glycodiversity in Animal



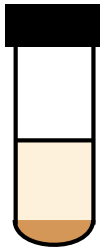
Strategies for deciphering complex glycoforms (non-GAG)



Starting Material
(tissues, cell pellets, biofluids)

Homogenization and organic extraction

Precipitate proteins by centrifugation



Supernatant
C/M/W(4/8/3)

Saponification

FOS, LLO

Reduction/alkylation
Trypsin digestion

Proteomics
Glycoproteomics
Site-mapping

Glycosphingolipids

Glycopeptides + Peptides

PNGaseF digestion

PNGaseF + ^{18}O-H_2O for N-linked site mapping

Mass spec.

Sep-pak tC18 clean-up

N-linked Glycans + Glycopeptides

Sep-pak Ion-exchange

Reductive β-elimination

FOS

LLO

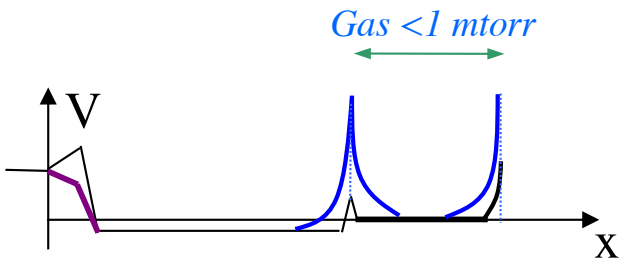
O-linked Glycans + Peptides

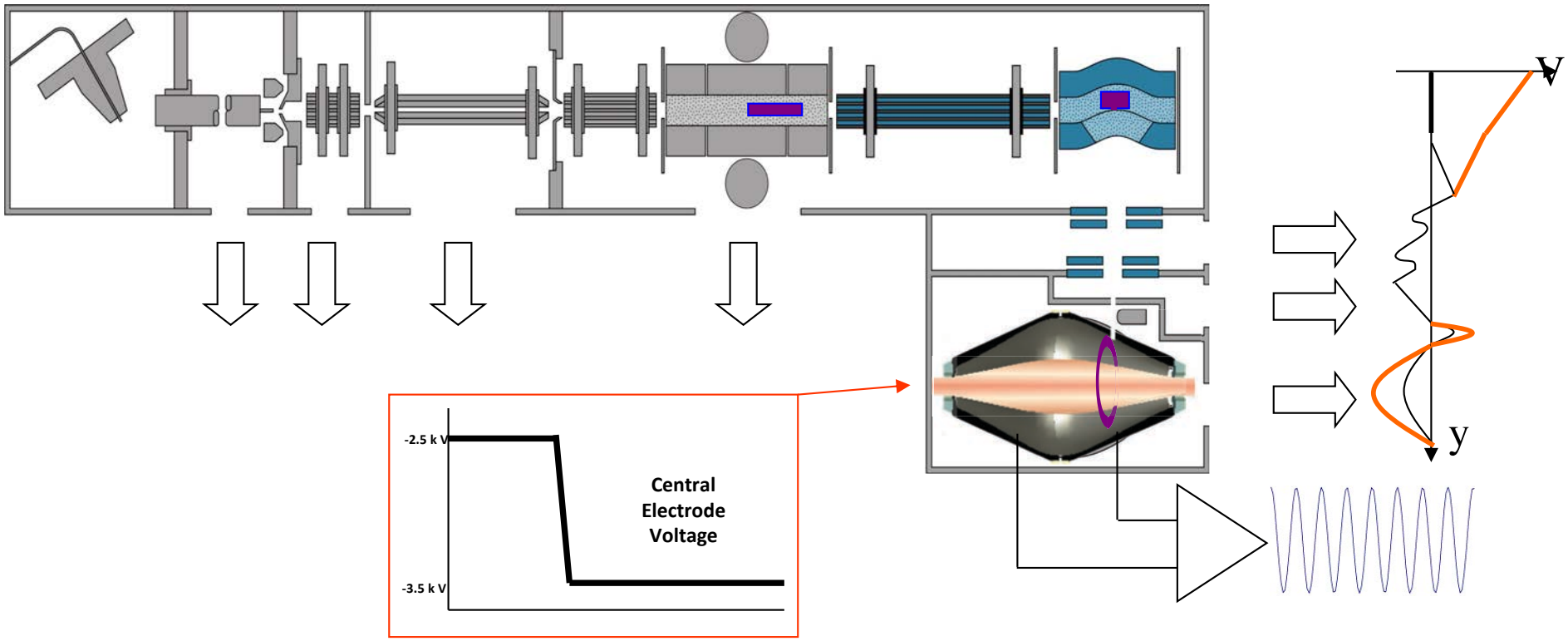
Permethylation for NSI-MS^n analysis of glycan structures

NSI-MS^n for glycan site utilization

LTQ-Orbitrap

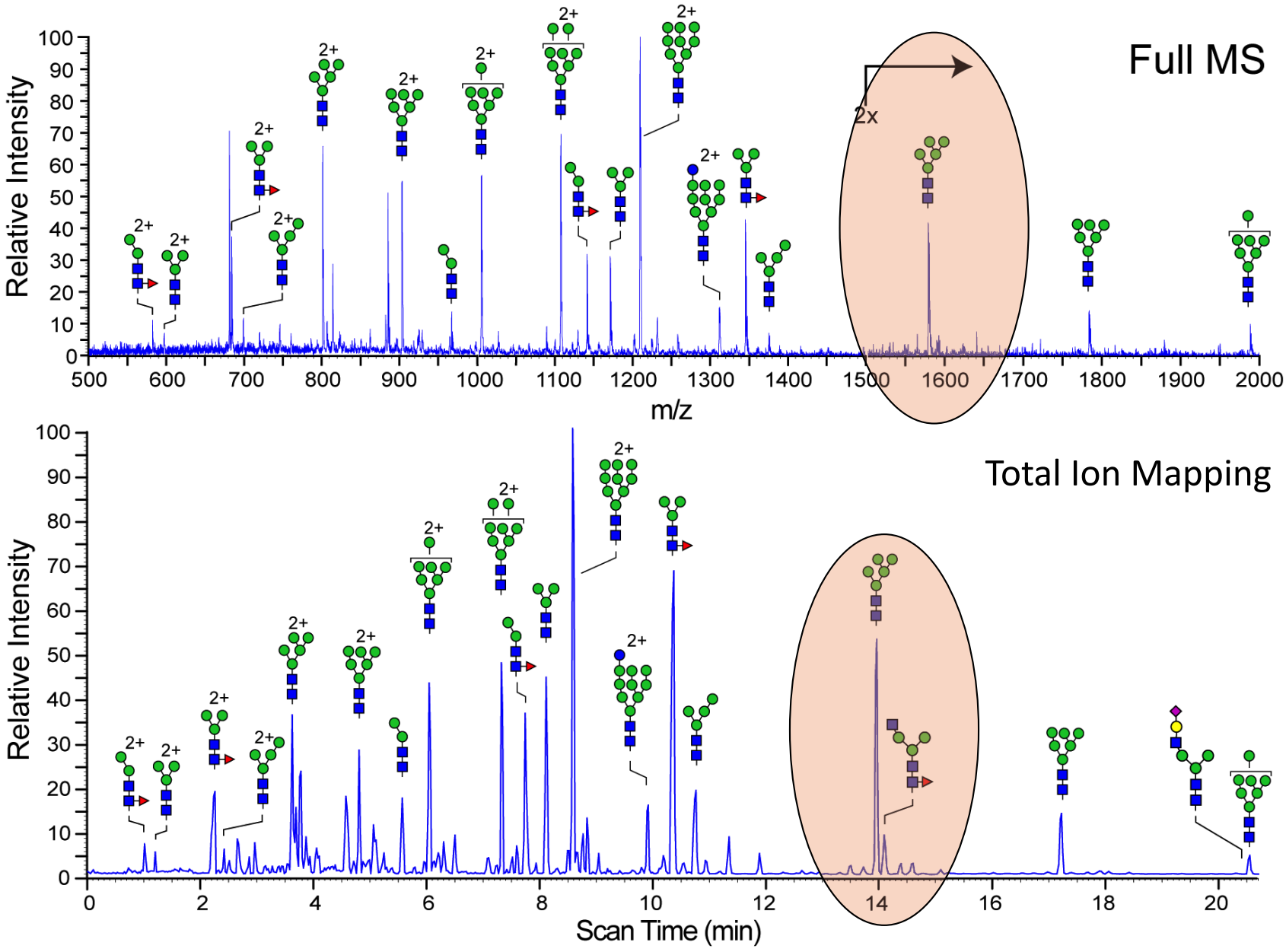
1. Ions are stored in the linear trap of LTQ
2. …are axially ejected
3. …and trapped in the C-trap and squeezed into a smaller cloud
4. …then a voltage pulse across C-trap ejects ions towards the Orbitrap
5. …where they are trapped and detected





Thermo Fisher Scientific

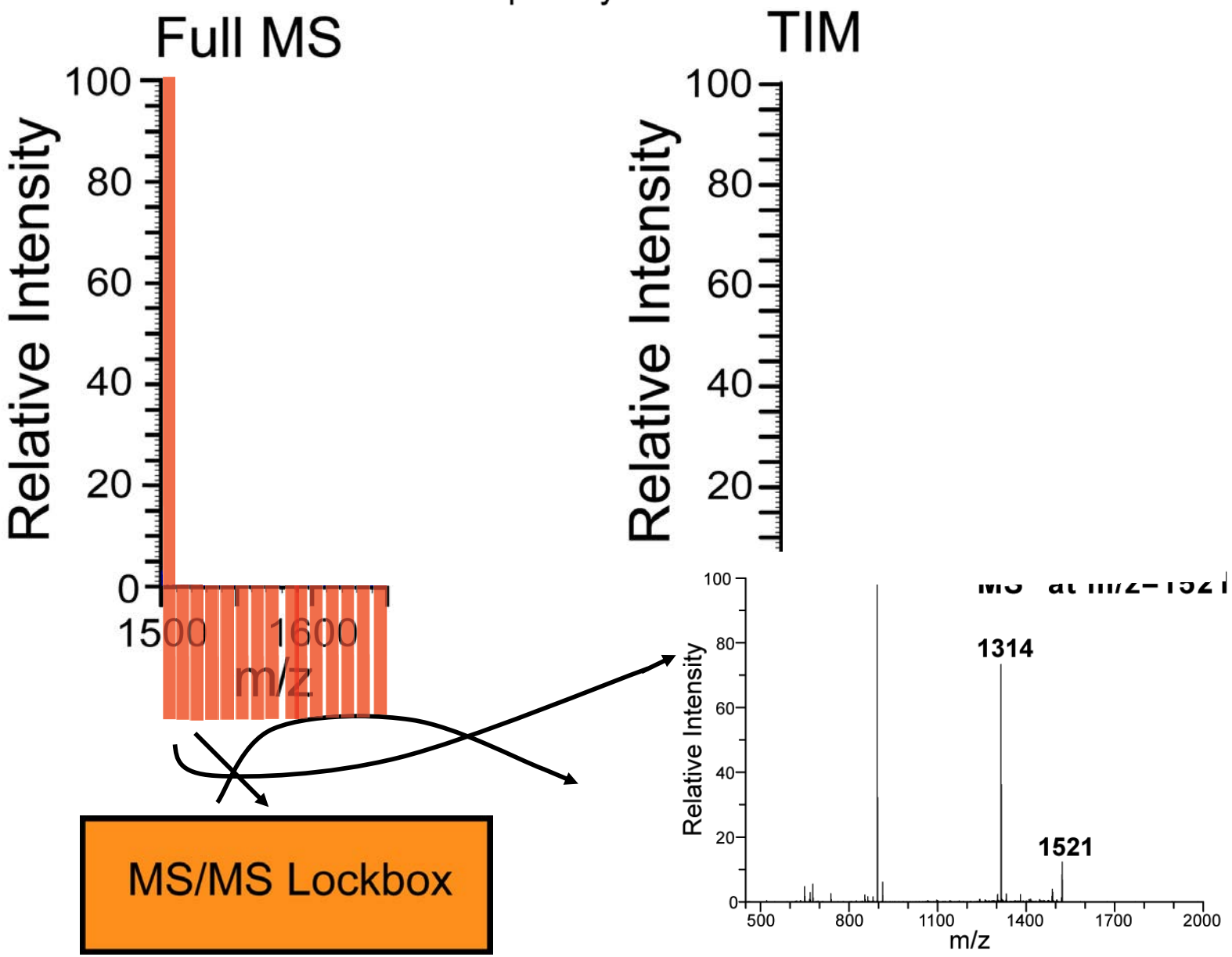
Total Ion Mapping (TIM) for relative quantification and enhanced detection of minor glycans



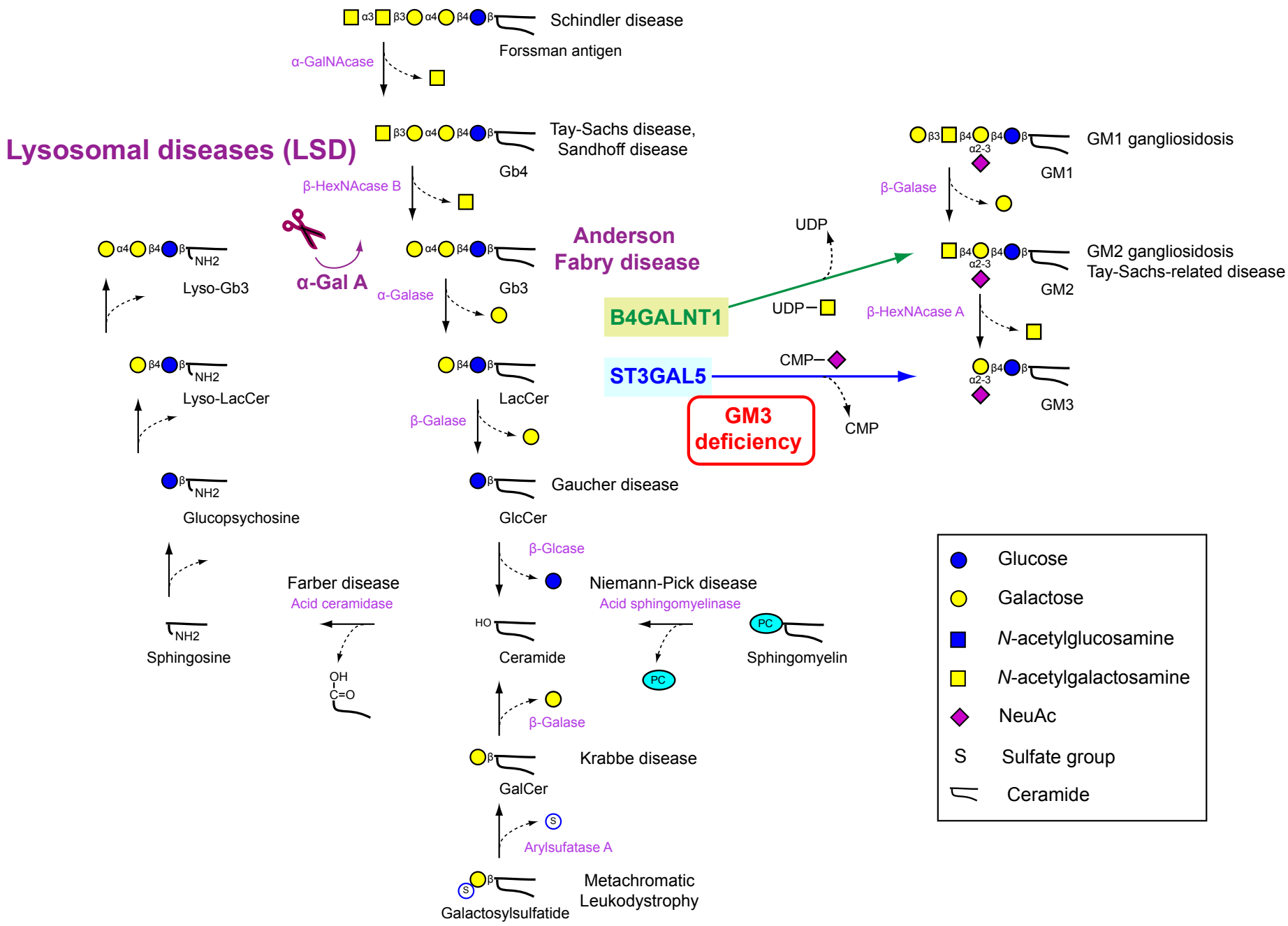
1. Trap ions within an acquisition window 2.8 mass units wide. Apply 28% collision energy, collect MS/MS spectrum, and plot TIC vs. scan time.

2. Store MS/MS fragmentation profile for future analysis.

3. Shift acquisition window by 2 mass units such that new window overlaps previoius window by 0.8 mass units. Repeat cycle.



Sphingolipid metabolism diseases



Glycosphingolipid

glycan + lipid called ceramide (Cer)

LacCer

GM3

GD3

SM3

3'OSO3

Ceramide (Cer)

Sphingosine

β4

O

OH

NH

C = O

R

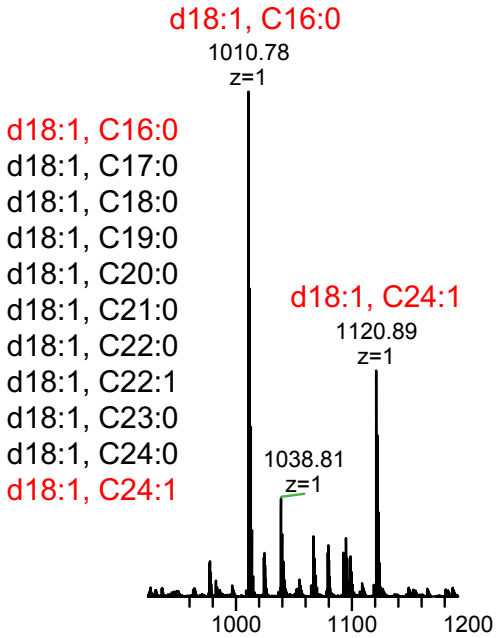
Fatty acid

Ceramide modification:
Length of alkyl chains,
OH, OAc, Lyso-type
desaturation, saturation

CGase
EGCase

Ozone (O_3)

Permethylated LacCer



GM3 synthase

+ CMP-

Old Amish infantile epilepsy syndrome
(GM3 synthase deficiency)

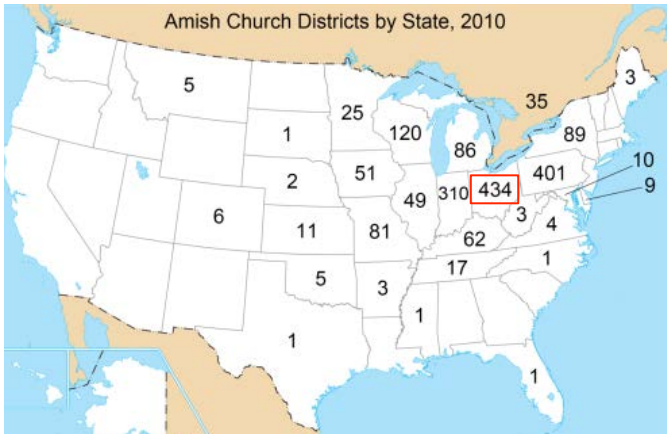
LETTERS

nature genetics

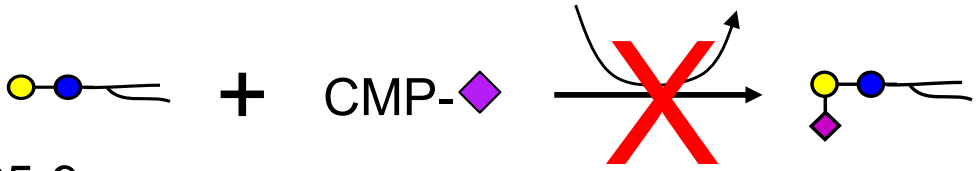
Infantile-onset symptomatic epilepsy syndrome caused by a homozygous loss-of-function mutation of GM3 synthase

Michael A Simpson[1], Harold Cross[2], Christos Proukakis[1], David A Priestman[3], David C A Neville[3], Gabriele Reinkensmeier[3], Heng Wang[4], Max Wiznitzer[5], Kay Gurtz[6], Argyro Verganelaki[1], Anna Pryde[1], Michael A Patton[1], Raymond A Dwek[3], Terry D Butters[3], Frances M Platt[3] & Andrew H Crosby[1]

www.nature.com/naturegenetics



- Identified from Ohio Amish communities.
- Loss of function of ST3GAL5 gene disrupts plasma ganglioside biosynthesis.
- Lack of GM3 and other complex gangliosides.
- Increase of LacCer.



Nat Genet. 2004, 36:1225-9.

Human Molecular Genetics, 2013 **1–16**
doi:10.1093/hmg/ddt434

A mutation in a ganglioside biosynthetic enzyme, *ST3GAL5*, results in salt & pepper syndrome, a neurocutaneous disorder with altered glycolipid and glycoprotein glycosylation

Luigi Boccuto[1,†], Kazuhiro Aoki[2,†], Heather Flanagan-Steet[2], Chin-Fu Chen[1], Xiang Fan[2], Frank Bartel[1], Marharyta Petukh[3], Ayla Pittman[1], Robert Saul[1], Alka Chaubey[1], Emil Alexov[3], Michael Tiemeyer[2,*], Richard Steet[2,*] and Charles E. Schwartz[1,*]

Identified from African-American siblings.

Identified a a missense mutation (p.E332K) mutation in the GM3 synthase gene.

European Journal of Human Genetics (2013) 21, 528–534

www.nature.com/ejhg

ARTICLE

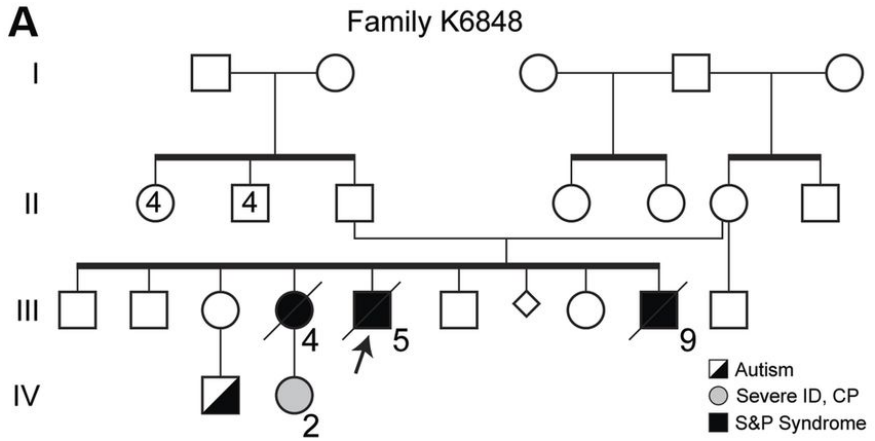
Refractory epilepsy and mitochondrial dysfunction due to GM3 synthase deficiency

Konstantina Fragaki[1,2,9], Samira Ait-El-Mkadem[1,2,9], Annabelle Chaussenot[1], Catherine Gire[3], Raymond Mengual[4], Laurent Bonesso[4], Marie Bénéteau[5], Jean-Ehrland Ricci[5], Valérie Desquiret-Dumas[6,7], Vincent Procaccio[6,7], Agnès Rötig[8] and Véronique Paquis-Flucklinger*[,1,2]

Identified from French cohorts.

Identified a single homozygous nonsense mutation (p.Arg288*) in the GM3 synthase gene.

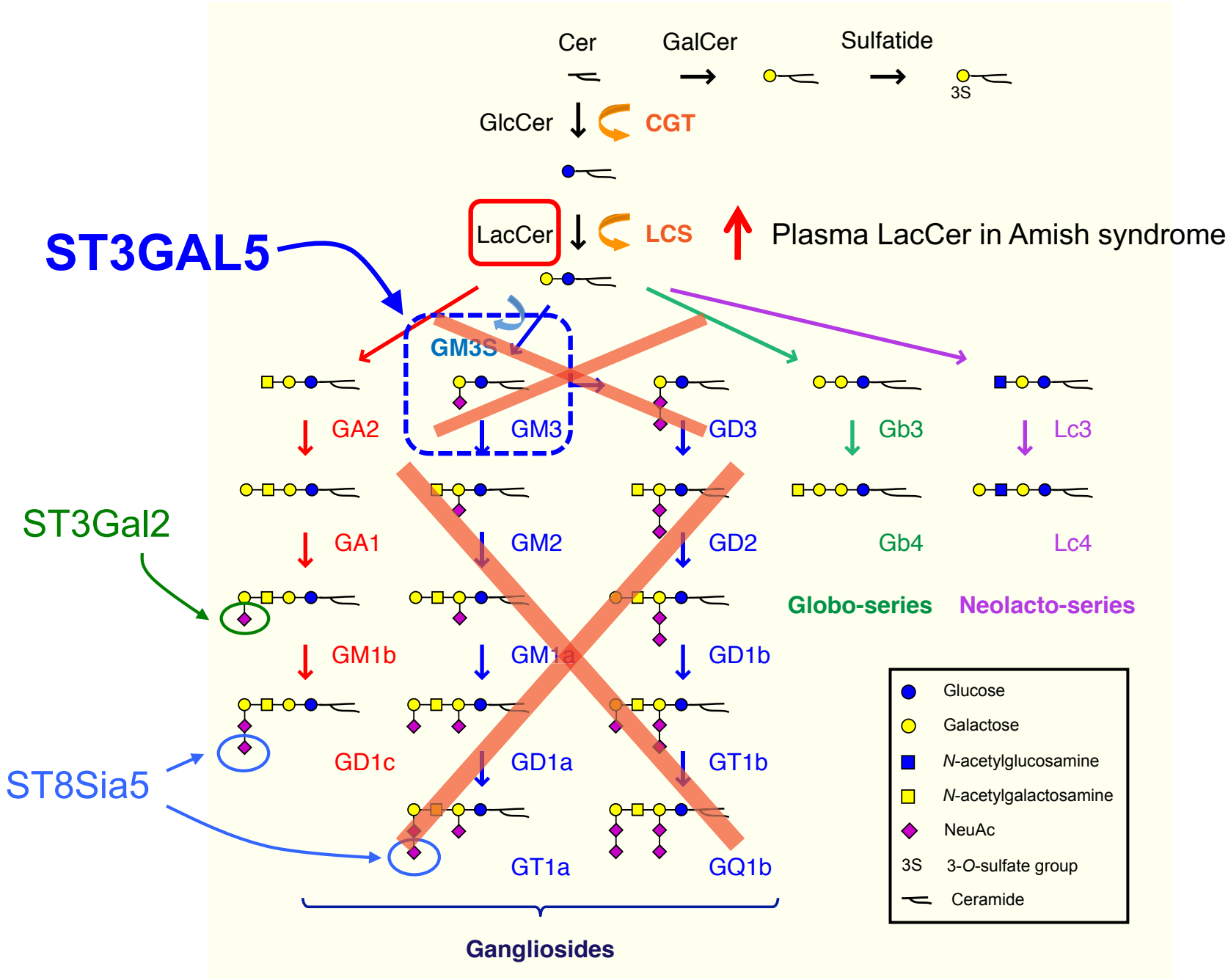
Salt and Pepper Syndrome: applying glycomic technology to investigating human disease mechanism



III-4 III-5 III-9

- In 1983, identified in 1 african-american family in the southeastern US at the Greenwood Genetic Center, Greenwood, SC
- Profound intellectual disability
- Failure to thrive
- Seizure disorder
- Midface hypoplasia
- Scattered dermal hyper- and hypopigmentation
- A missense mutation (p.E332K) was identified in the ST3GAL5 (GM3 synthase) gene of two siblings.
- This newly identified syndrome is allelic to Old Amish infantile epilepsy syndrome.

Altered GSL biosynthesis in GM3 deficiency



Allelic to a disorder described in Ohio and Pennsylvania Amish communities (caucasian)

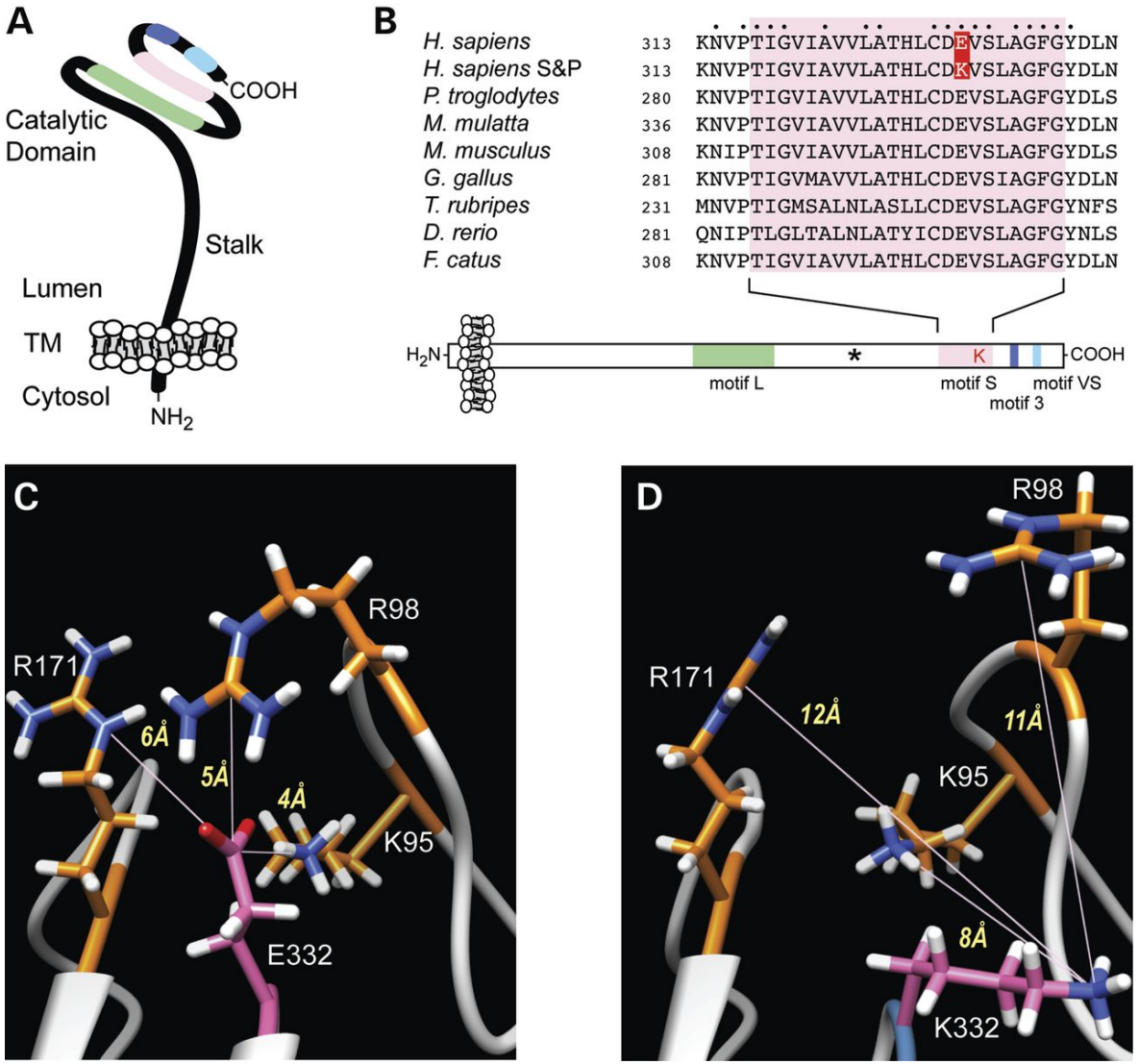
Salt and Pepper Syndrome

Old Amish infantile epilepsy syndrome

Salt and Pepper Syndrome: missense mutation (E332K) in ST3GalV (GM3 Synthase); Amish syndrome: truncation (R232X) in ST3GalV

Saul et al. Proc. Greenwood Genetic Center, 2:6-9, 1983; Simpson, M., et al., (2004) Nature Genetics 36, 1225

The ST3GAL5 mutation in S&P syndrome generates a p.E322K missense mutation within a highly conserved sialyltransferase domain.



Hum. Mol. Genet. 2013;hmg.ddt434

Human Molecular Genetics

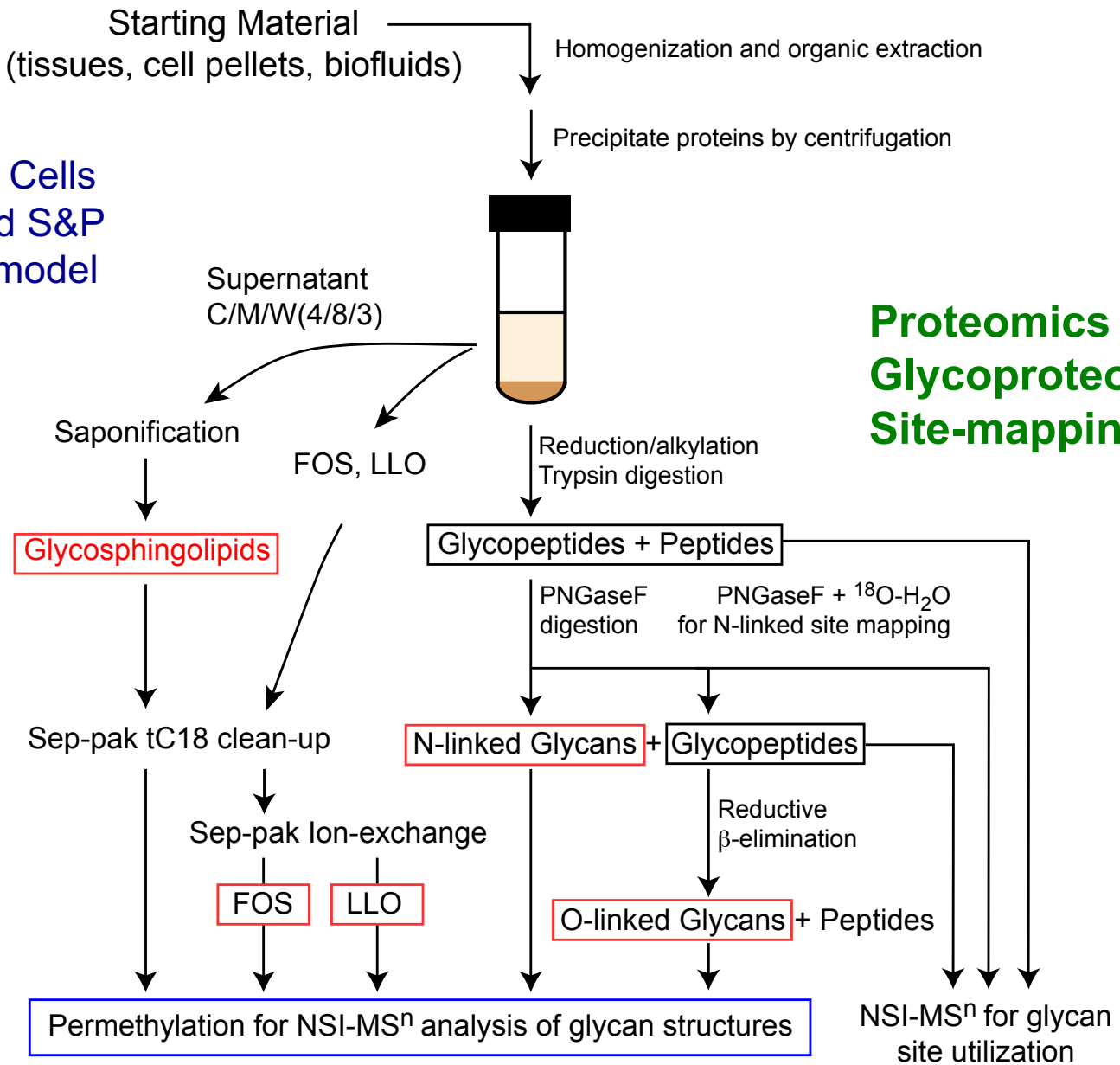
Does a single mutation of st3gal5 gene impact on other glycosylation machinery?

Applying MS-based comprehensive glycomics to investigate the disease mechanism

GM3 deficiency

Fibroblast, Neural Crest Cells derived from Control and S&P iPS cells and zebrafish model

Mass spec.

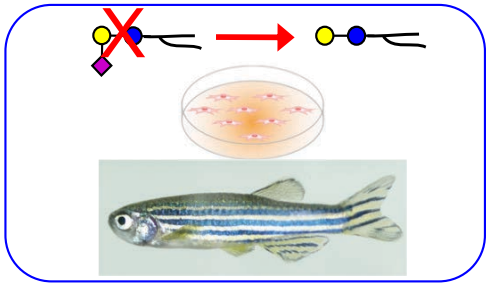


Proteomics
Glycoproteomics
Site-mapping

Human Salt and Pepper fibroblasts compared to Zebrafish morpholino ST3Gal5 model

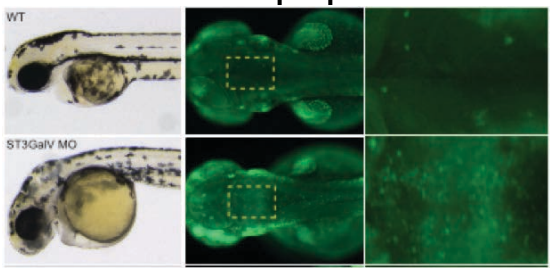
- Human fibroblasts show greater loss of GM3 (functional null) than detected in Zebrafish morpholino knockdown.

Lack of GM3



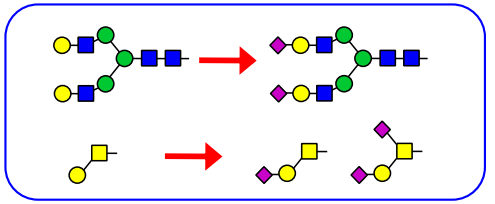
- Zebrafish phenotype indicates neural cell death is increased when GM3 and other complex gangliosides are decreased.

Neural apoptosis



- Zebrafish model also demonstrates increase of glycoprotein sialylation.

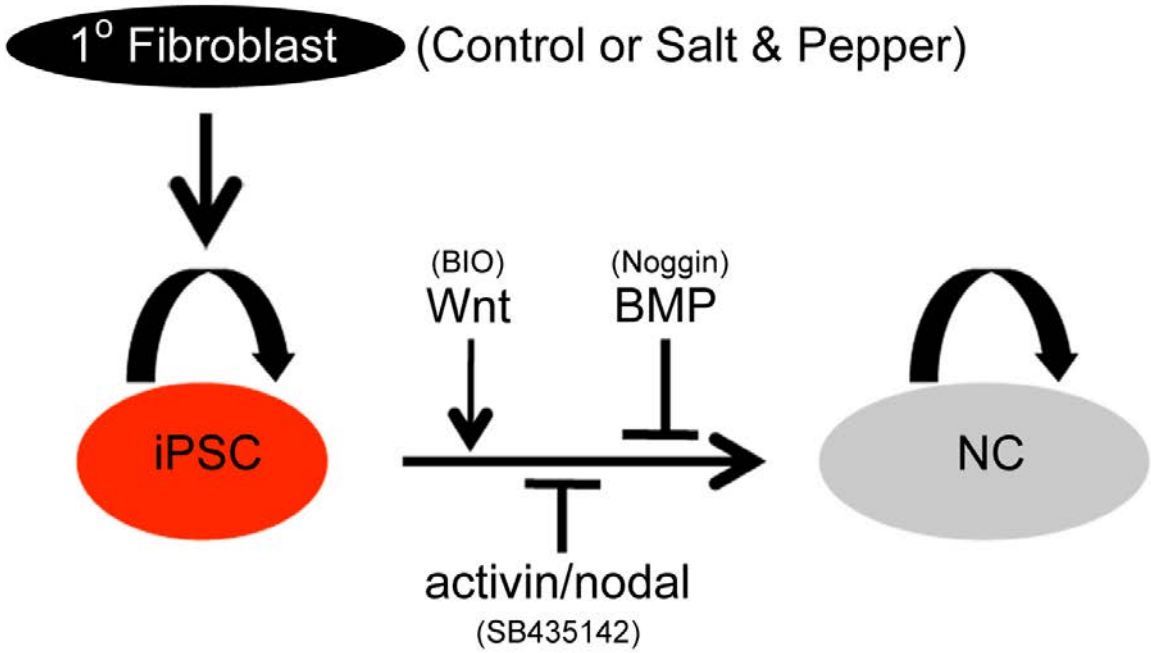
More sialylation

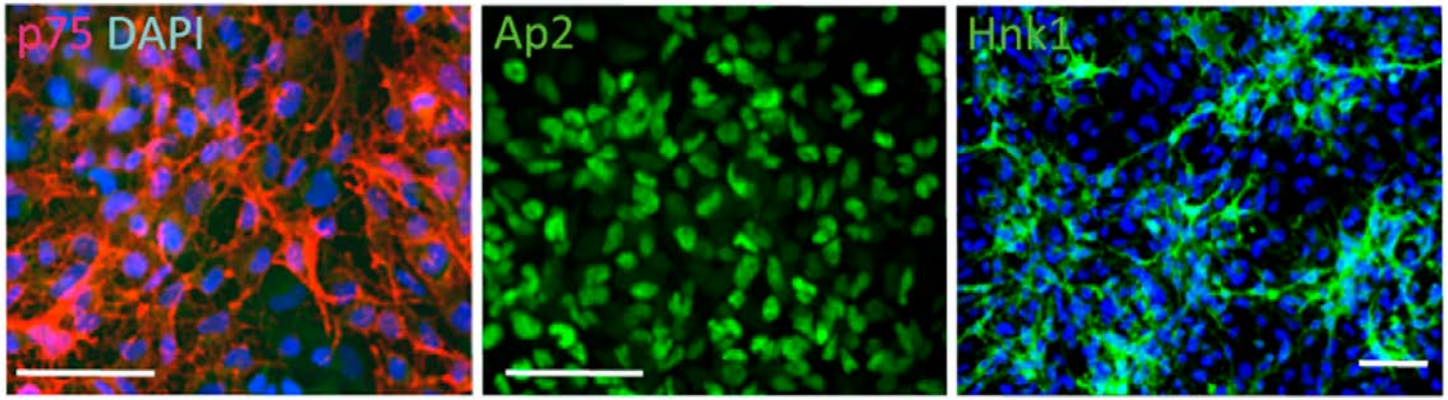


- Would be useful to investigate this in neural cells derived from human Salt and Pepper or Amish populations.

Hum Mol Genet. 2014, 23:418-33. Boccuto L, Aoki K, et al.

Generation of induced pluripotent cells from S&P fibroblasts



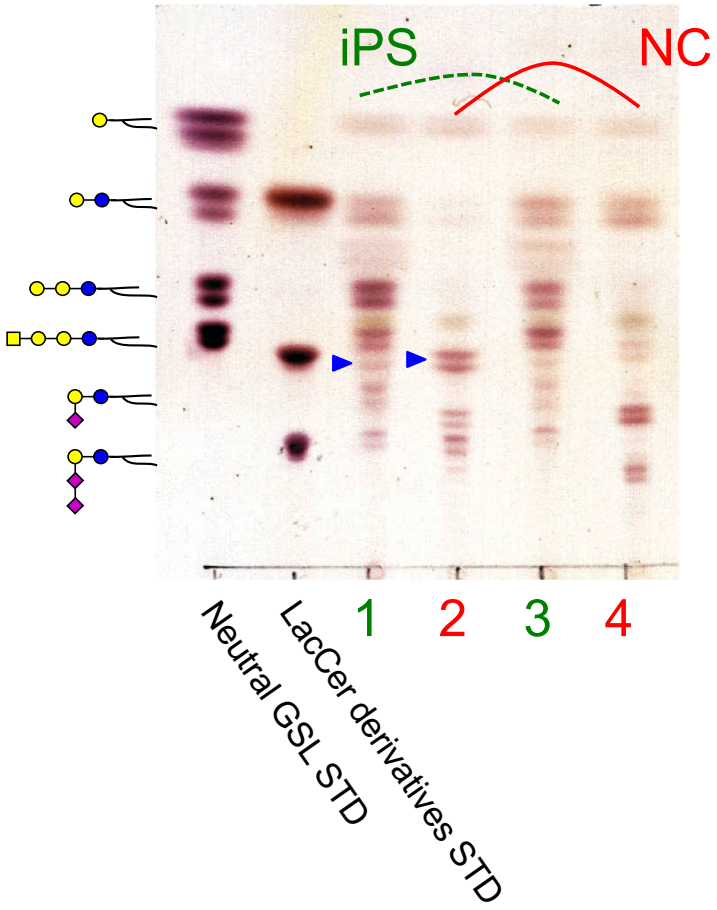


Also, negative for Sox2, Pax6, Oct4, Nanog

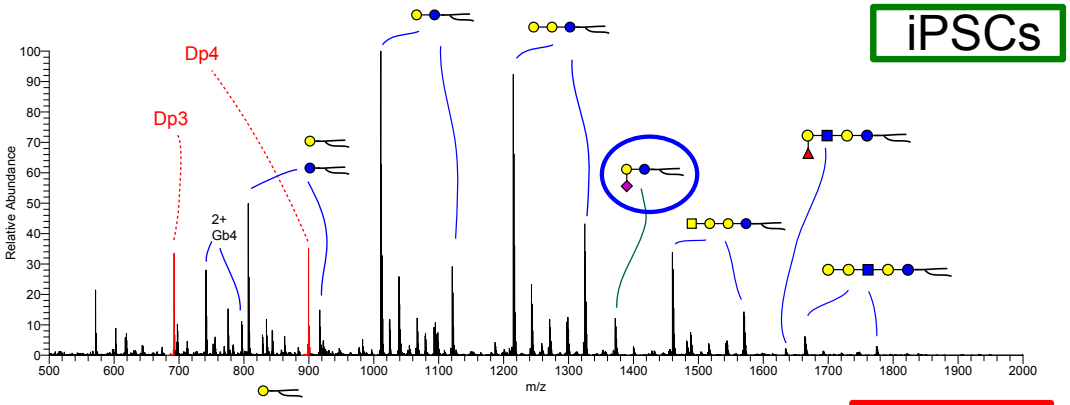
Laura Menendeza, Steve Dalton. Proc Natl Acad Sci. (2011) 108:19240

NSI-MS profiles

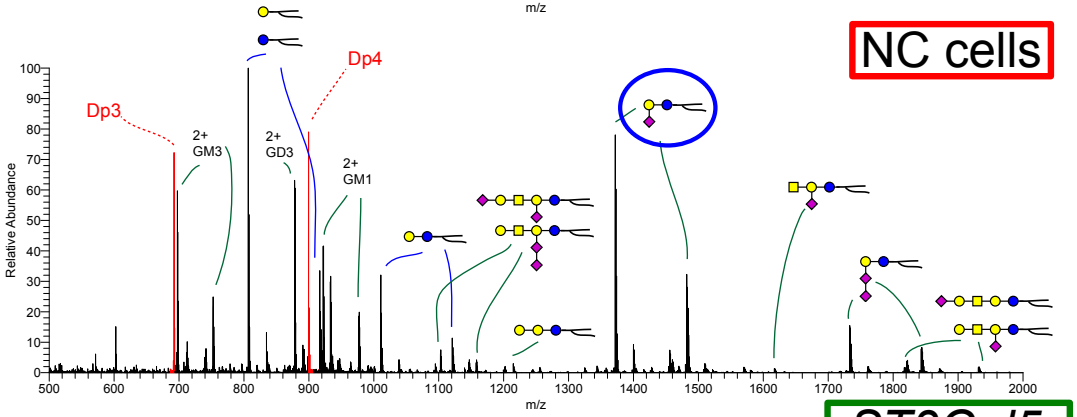
TLC



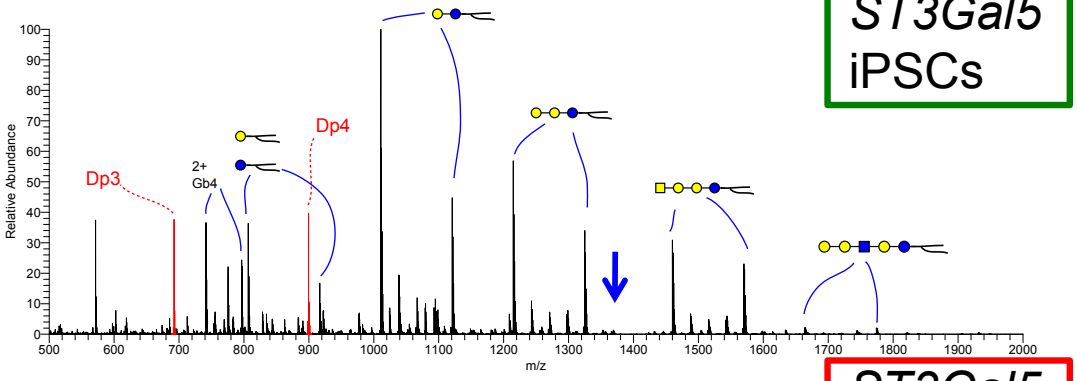
1



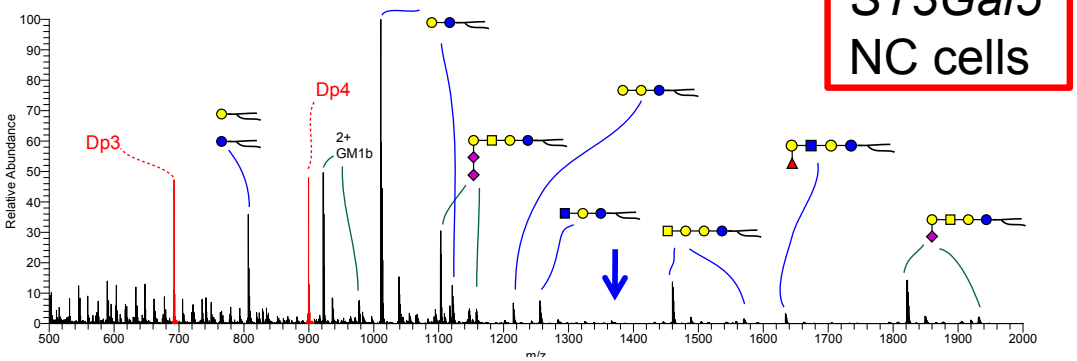
2



3



4



Pennsylvania Amish

Clinic for Special Children

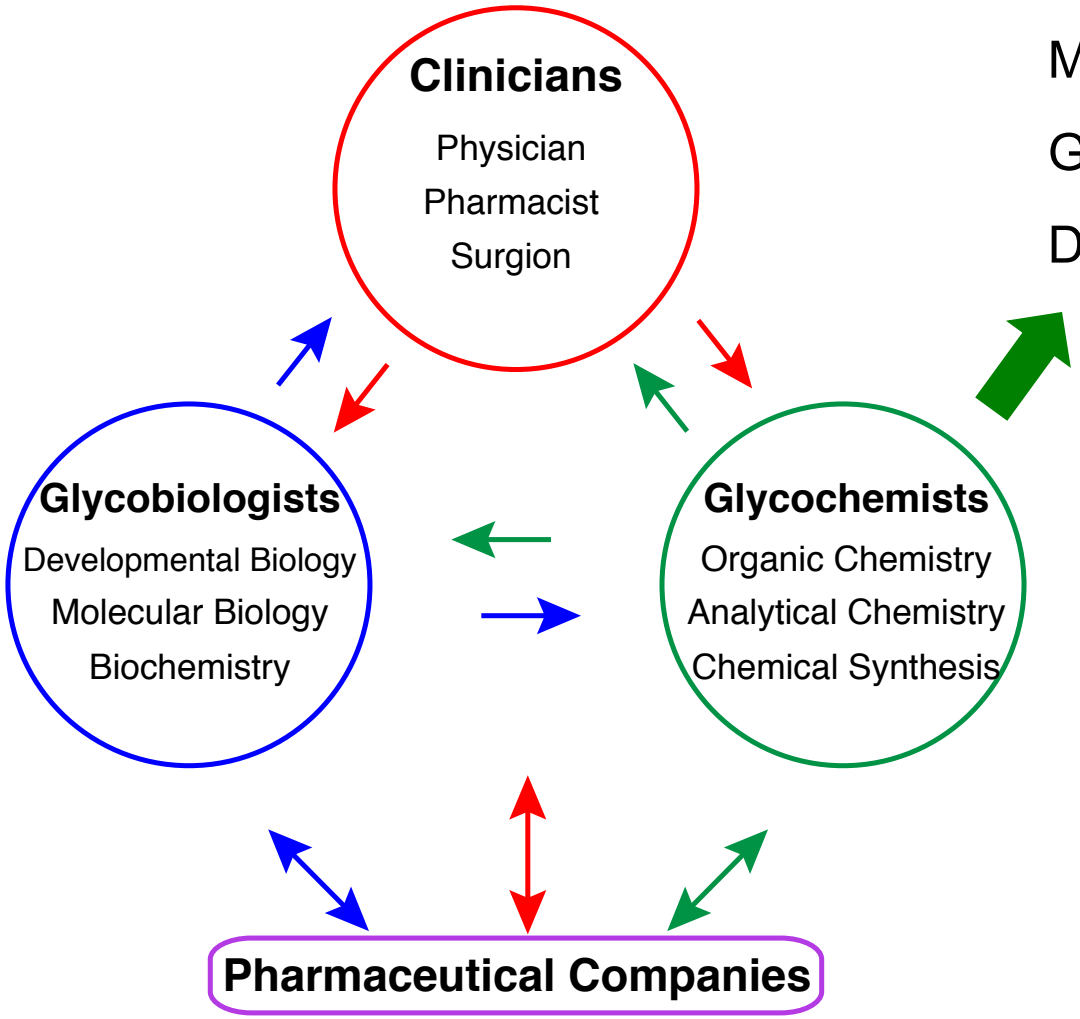
535 Bunker Hill Rd. Strasburg PA 17579; Phone 717-687-9407. Fax 717-687-9237
www.clinicforspecialchildren.org

Erik Puffenberger, Kevin Strauss and Holmes Morton (from left to right) in front of the Clinic for Special Children, which treats those with inherited disorders.

Genomics, plain and simple

A Pennsylvania clinic working with Amish and Mennonite communities could be a model for personalized medicine.

Linking to human health and disease



Method development

Glycome profiling

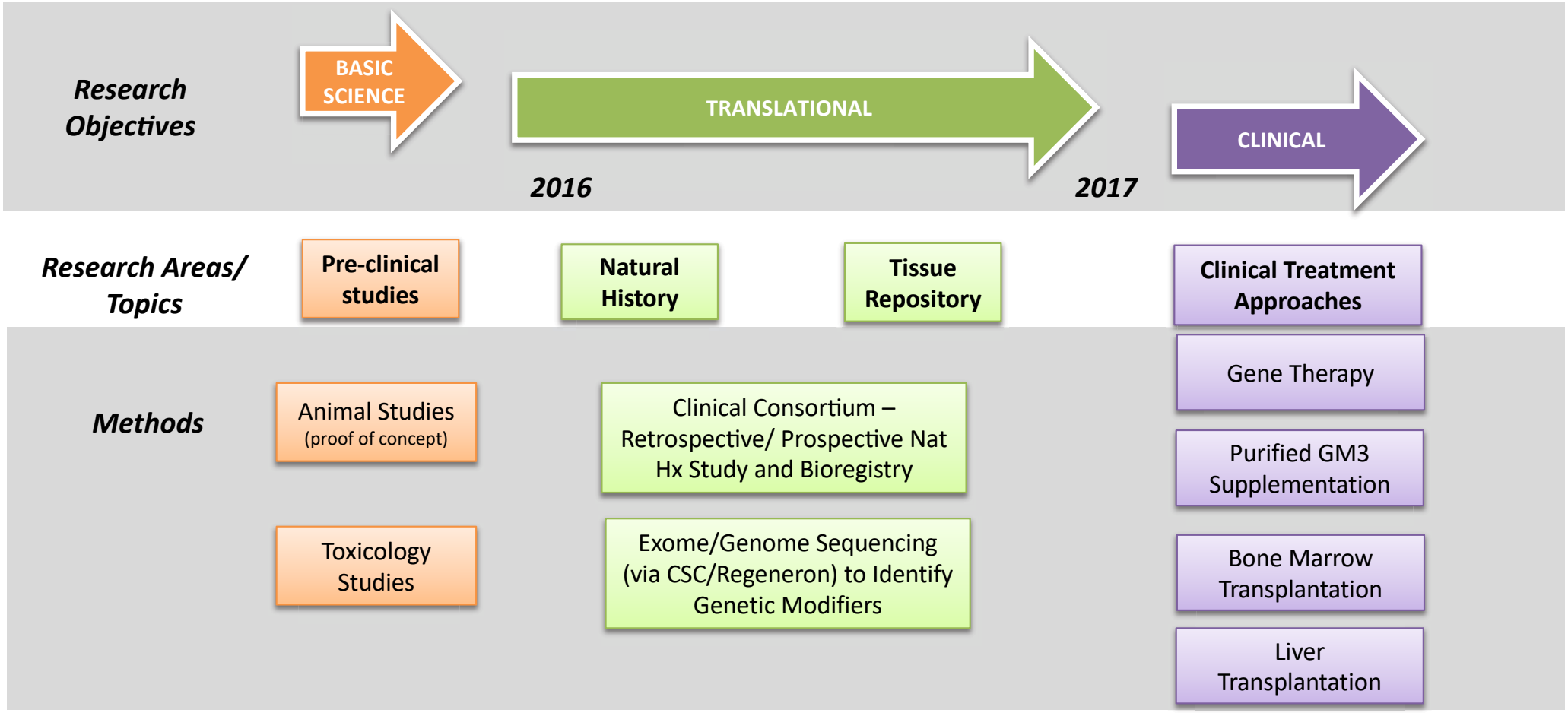
Data analysis

With strong support of bioinformatics group

center for rare disease therapy

GM3 Synthase Deficiency Research Roadmap to a Cure

Children's Hospital of Pittsburgh of UPMC



Umbilical Cord blood stem cell transplant

Connor Mast, DOB 10/27/2015

Clinic *for* Special Children

Patient | Notes and Alerts | Providers | Map | Scanned Records

Patient Number	3101	Account Number	2271
First	Connor		
Last	Mast		
Married surname			
DOB	10/27/2015		
Sex	Male		
SSN			
LGH MR#			
Nemours MR#			
Presenting diagnosis	GM3 synthase deficiency		
Diagnosis 2			
Affiliation			
MolDx			

Primary	Dale Mast
DOB	3/18/1971
SSN	
Secondary	Crystal (Toms) Mast
DOB	9/27/1976
SSN	
Address	
City, State, Zip	
Telephone 1	
Telephone 2	
Telephone 3	
E-mail	

Medical Problems

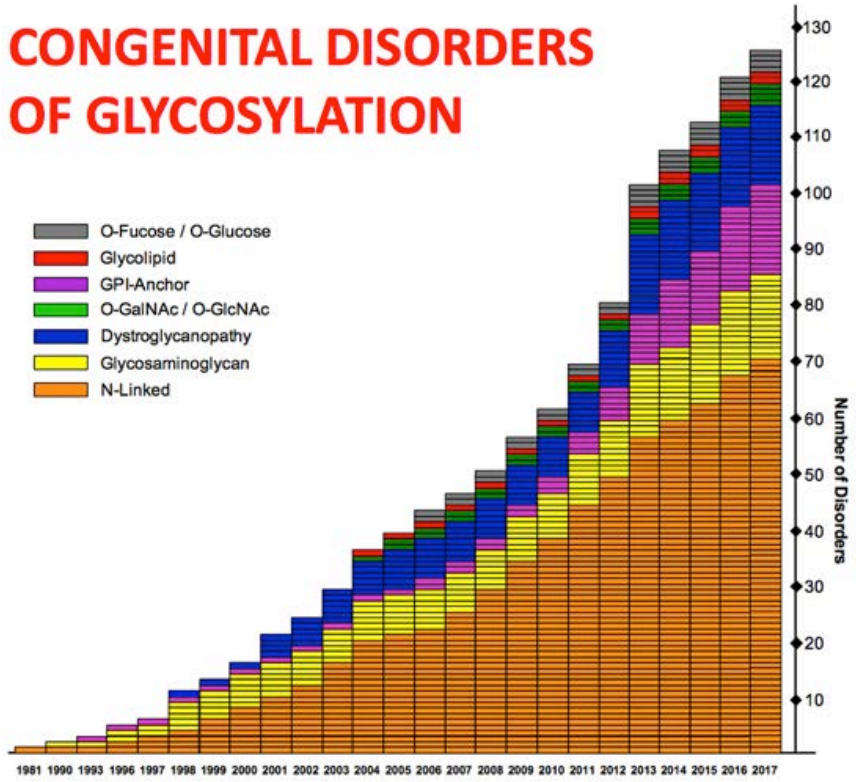
HPO Phenotypes

Surgery at 6months old

Paul Szabolcs, MD

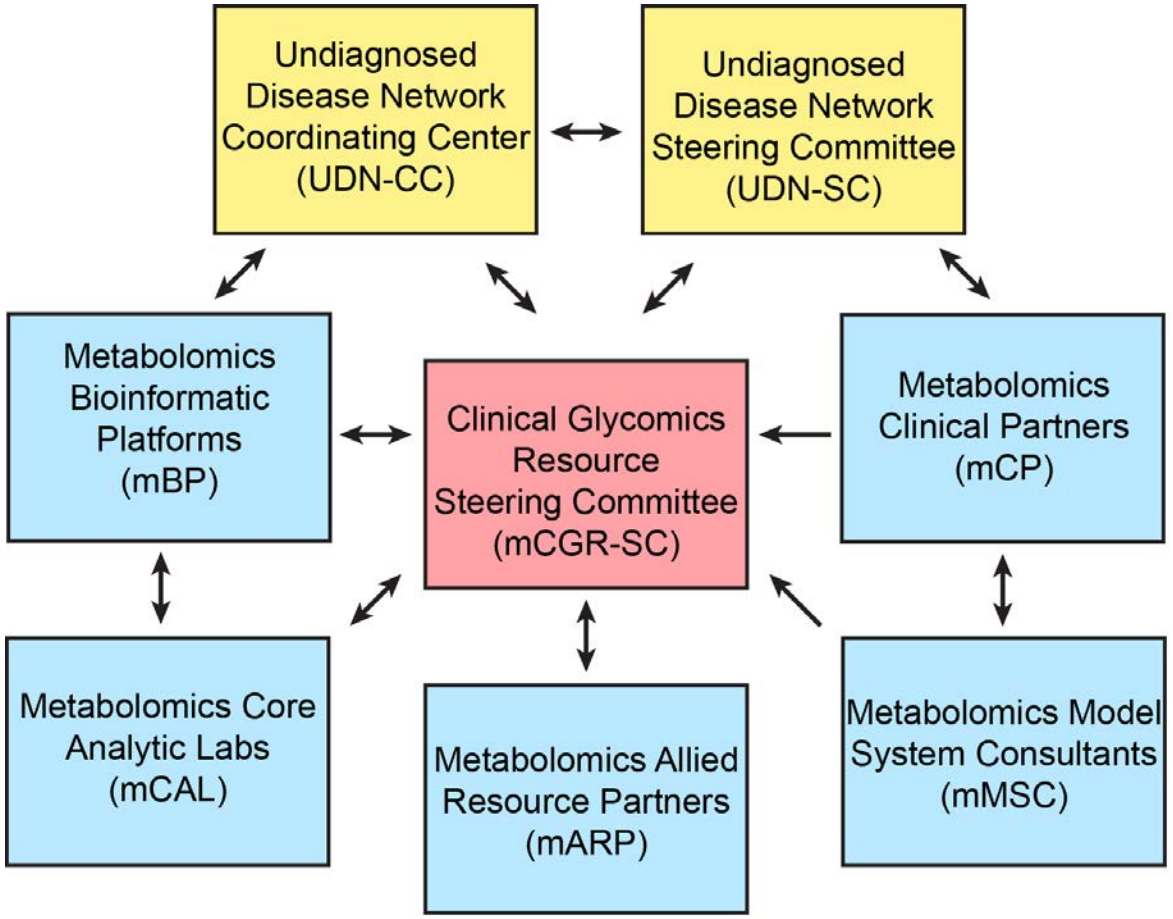
University of Pittsburgh School of MedicineChief,
Division of Blood and Marrow Transplantation and Cellular Therapies,
Children's Hospital of Pittsburgh of UPMC

Increasing identification of human disorders that affect glycosylation



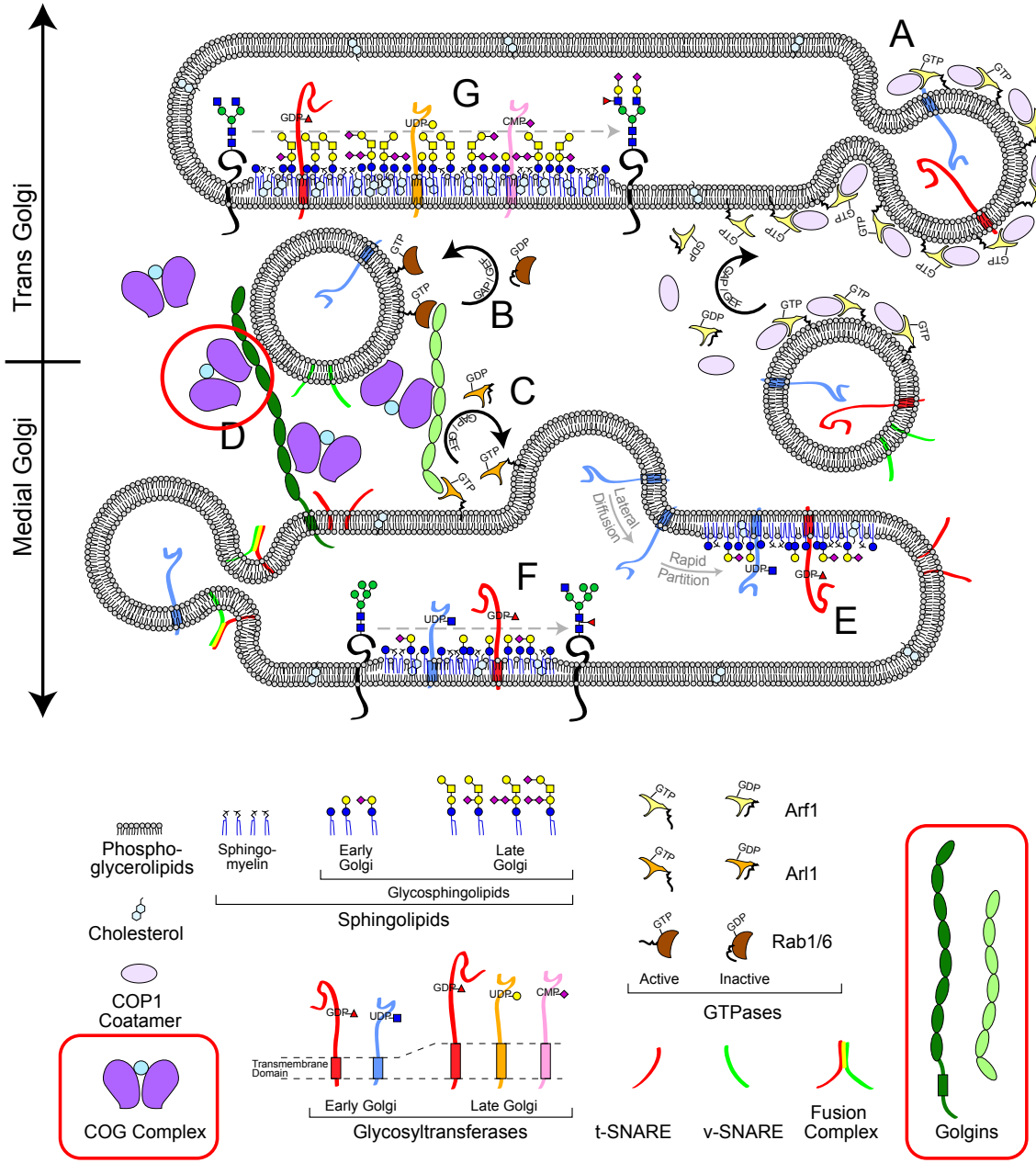
We are applying our glycomic technologies for deciphering disease mechanisms in undiagnosed disorders.

Establishment of Clinical Glycomic Resource for Characterizing Undiagnosed Diseases



Hum Mol Genet. 2009 Sep 1;18(17):3244-56.
Golgi function and dysfunction in the first COG4-deficient CDG type II patient.
Reynders E, et al.

Analysis of COG4-deficient zebrafish (CDG type II)

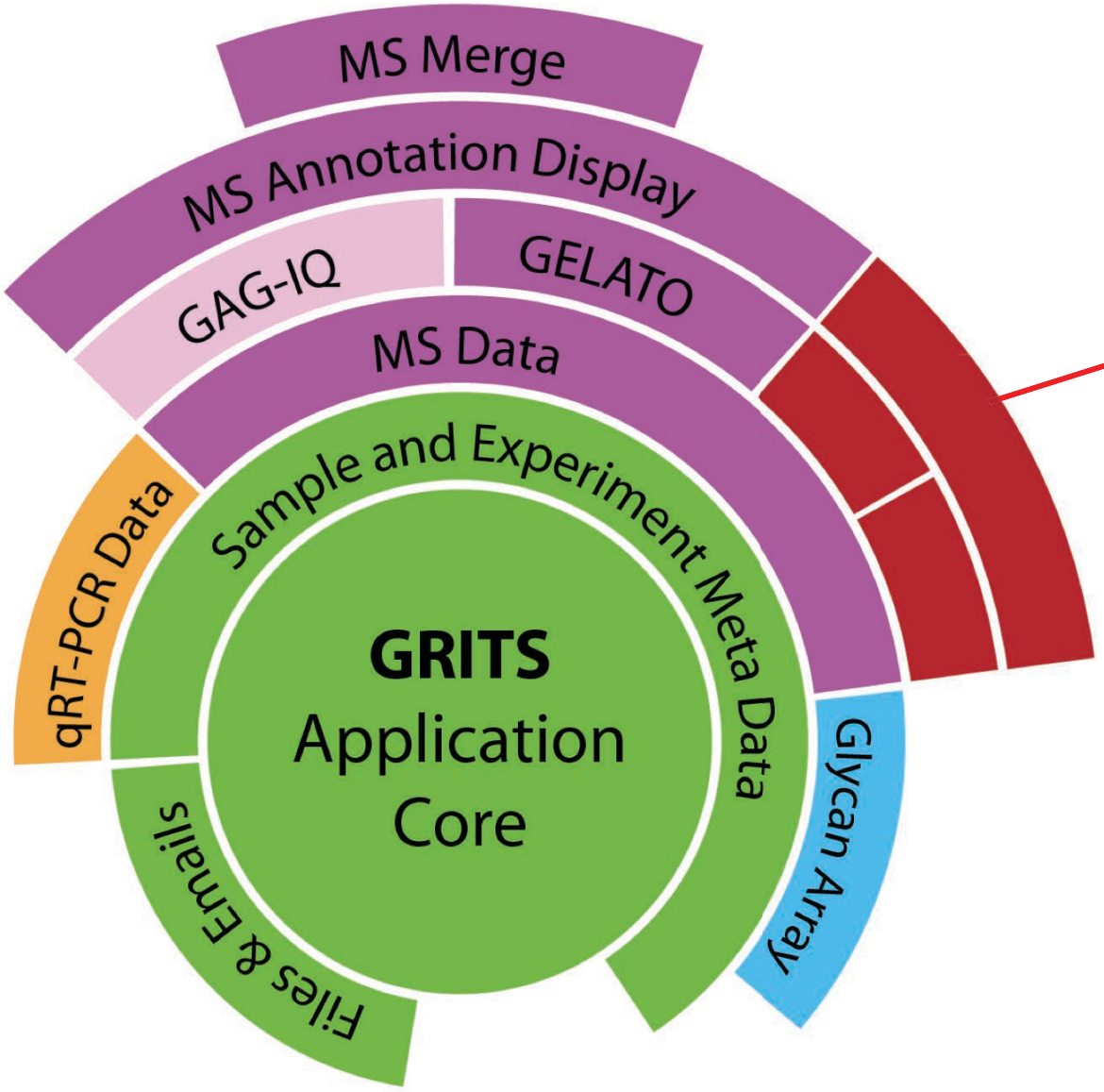


The spectrum of mass spectrometry for glycomics

Type of analysis	Expertise required	Throughput	Pros	Cons	Useful for
MS	✓	High	Fastest	Least structural information	First pass analysis of spectral complexity
MS/MS with manual interpretation	✓✓✓✓	Low	Yields structural topology	Slow, requires significant expertise	Comparison of small sample number
MS/MS with automated interpretation	✓✓	High	Yields structural topology and high throughput	Requires highly curated, non-redundant database	Comparison of many samples
MS^n	✓✓✓✓✓✓	Extremely Low	Yields greatest amount of structural information	Slow, requires high level of expertise	Discovering novel glycans, focused analysis on small sample size

Although interpretation of MS and MS/MS data builds great camaraderie among graduate students, post-docs, and faculty….

An extendible software platform for the processing and archiving of glycomics data



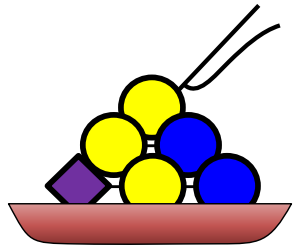
New plugins for processing and analysis of **glycolipid MS data** as common funds Projects (R21, PIs: KA&RR)

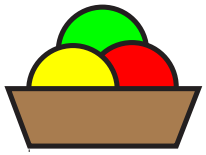
René Ranzinger

NIH The Common Fund

DANGO: An MS **D**ata **An**notation Systems for **G**lycolipid-**o**mics

- Semi-automated annotation software for glycolipids
  - Support high throughput MS data analysis
  - Reduce the time required for annotation
- Development as a part of GRITS-Toolbox
  - Reuse GELATO systems for glycan annotation
  - Implement calculation systems for lipid from scratch
  - Annotate glycolipids as combination of glycan and lipid

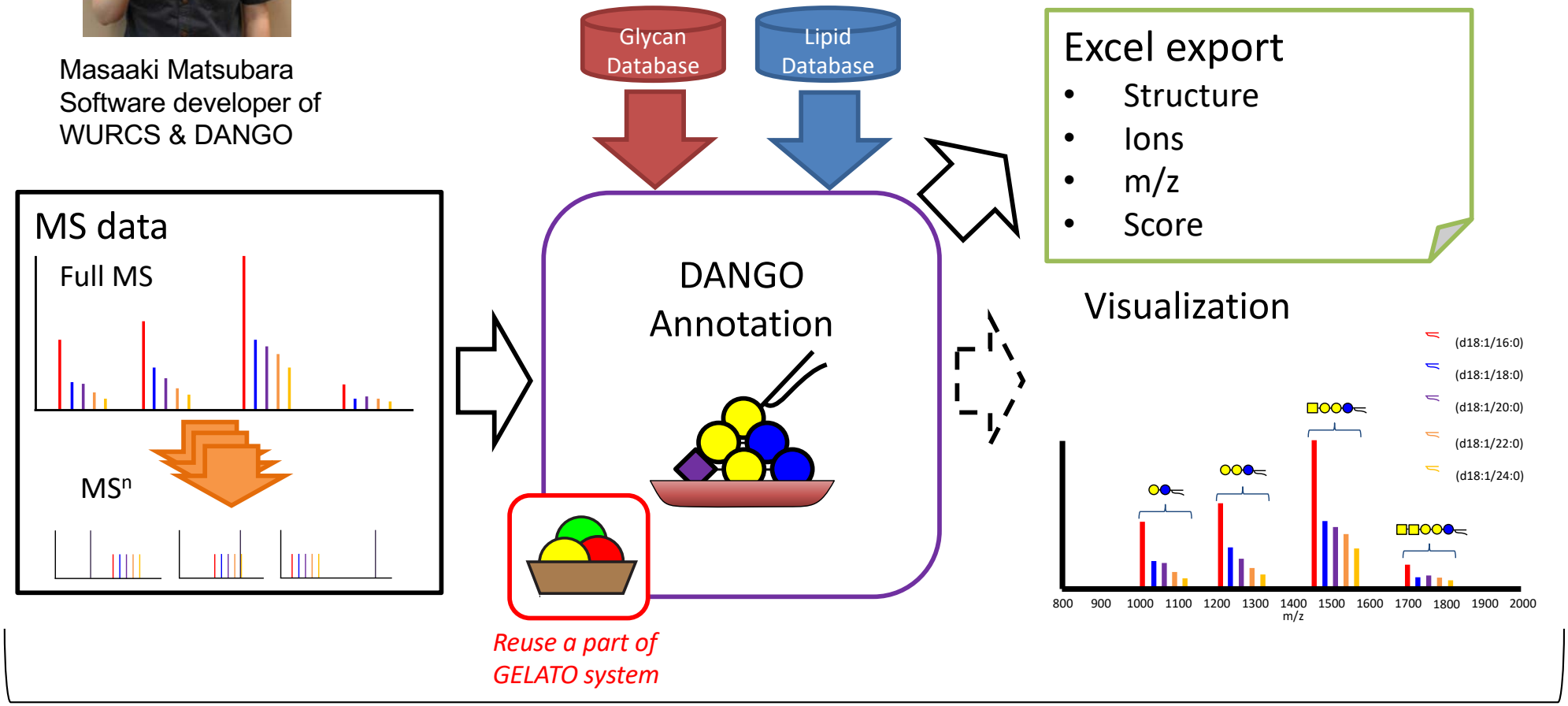




DANGO annotation workflow

Masaaki Matsubara
Software developer of
WURCS & DANGO

Mayumi Ishihara
GSL analyst



GRITS Toolbox

How to generate glycan database, tools and the web resources

Glycan class

N-glycan

O-glycan

GSL

GAG

FOS

LLO

GPI

Other complex glycans

Species/cell type specific glycans

Human derived samples
Biofluids (serum, RBC), cultured cells, tissues, etc

Model organisms
Drosophila, zebrafish, worm, yeast, slim mold, etc

Other living organisms
Sturgeon, dolphin, cat fish, salmon, sea turtle, soft-shell turtle, coyote, raccoon, bobcat, ring-neck duck, wild pig, opossum, frog, alligator, etc

In collaboration with

Georgia Aquarium
Savannah River Ecology Laboratory
Dr. Tadashi Suzuki, RIKEN

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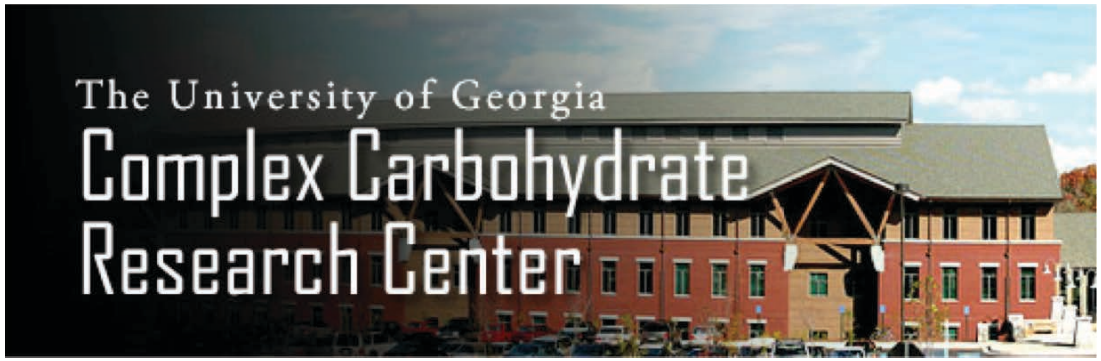

The Complex Carbohydrate Research Center

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Richard Steet, CCRC (zebrafish)
Michael Kulik, UGA (stem cell)
Stephen Dalton, UGA (stem cell)
Luigi Boccuto, Greenwood Genetic Center, SC (S&P)
Charles Schwartz, Greenwood Genetic Center, SC (S&P)
SREL, SC
GA Aquarium, GA
Tadashi Suzuki, RIKEN

Integrated Technology Resource for Biomedical Glycomics